

MegaMan Human Transcriptome Library

INSTRUCTION MANUAL

Catalog #790000 MegaMan Human Transcriptome Library (20 reactions)

#790001 MegaMan Human Transcriptome Library (100 reactions)

Revision A.01

For In Vitro Use Only

790000-12

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Agilent Technologies

Stratagene Products Division

11011 North Torrey Pines Road

La Jolla, CA 92037

Telephone (858) 373-6300

Order Toll Free (800) 424-5444

Technical Services (800) 894-1304

Internet techservices@agilent.com

World Wide Web www.stratagene.com

Europe

Location	Telephone	Fax	Technical Services
Austria	0800 292 499	0800 292 496	0800 292 498
Belgium	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 15775	0800 15740	0800 15720
France	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 919 288	0800 919 287	0800 919 289
Germany	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 182 8232	0800 182 8231	0800 182 8234
Netherlands	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 023 0446	+31 (0)20 312 5700	0800 023 0448
Switzerland	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 563 080	0800 563 082	0800 563 081
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 917 3282	0800 917 3283	0800 917 3281

All Other Countries

Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.

MegaMan Human Transcriptome Library

CONTENTS

Materials Provided	1
Storage Conditions	1
Additional Materials Required	1
Introduction	2
Protocol	5
PCR Protocol.....	5
Reaction Amplification	6
Expected Control Amplification Results	6
Troubleshooting	6
MSDS Information	6
Quick Reference Protocol	8

MegaMan Human Transcriptome Library

MATERIALS PROVIDED

MegaMan Human Transcriptome Library (Catalog # 790000)

Materials provided	Concentration	Quantity
MegaMan Library	160 ng DNA/ μ l	20 μ l
Control Primer Set forward primer 5' AATGTGCATGTTCCAGCTGC 3' reverse primer 5' AAAGAGCAGGCTCTGTACTC 3'	10 μ M of each	10 μ l

MegaMan Human Transcriptome Library (Catalog # 790001)

Materials provided	Concentration	Quantity
MegaMan Library	160 ng DNA/ μ l	100 μ l
Control Primer Set forward primer 5' AATGTGCATGTTCCAGCTGC 3' reverse primer 5' AAAGAGCAGGCTCTGTACTC 3'	10 μ M of each	10 μ l

STORAGE CONDITIONS

All Reagents: -20°C

ADDITIONAL MATERIALS REQUIRED

Acetamide

DMSO

DNA Polymerase

Recommended: *PfuUltra* DNA Polymerase [Stratagene Catalog #600380 (100 U), #600382 (500 U), and #600384 (1000 U)]

Temperature cycler

PCR tubes^{||}

PCR primers

Deoxynucleoside triphosphates (dNTPs)

NOTICES TO PURCHASER

For use in PCR amplification only.

Manufactured for Agilent by Genofi.

^{||} Thin-walled PCR tubes are highly recommended for use with Stratagene thermal cyclers. These PCR tubes are optimized to ensure ideal contact with the multiblock design to permit more efficient heat transfer and to maximize thermal-cycling performance.

INTRODUCTION

The Stratagene MegaMan human transcriptome library is a collection of cDNA created using mRNA from 66 diverse sources, including 32 from human tissues and 34 from human cancer cell lines (Table 1). This human transcriptome library is ideal for cloning both well-characterized genes and transcripts previously identified by expressed sequence tag (EST) studies. Additionally, this library can be used for the validation of predicted genes and alternative splice isoforms generated from The Human Genome sequencing data. The library contains full-length cDNA sequences, providing for downstream applications, and provides a quick screen for gene validation and discovery.

The MegaMan human transcriptome library is the largest comprehensive template of the human transcriptome in a single tube, and can be used in an extensive variety of cloning projects. This library is a complex mixture of high-quality plasmid libraries representing tens of millions of primary clones (Figure 1A). In order to maximize the probability of good representation of even the rarest, longest and most fragile transcripts, the construction of these libraries includes an enrichment protocol which singles out and pools the longest cDNAs. Further, because adapters are not ligated onto the ends of the cDNA, genomic DNA false inserts as well as fusion cDNAs from two different genes are nearly nonexistent.

In order to construct the MegaMan human transcriptome library, each individual library is size fractionated, and each fraction (low-sizing: >800 bp and high-sizing: >2000 bp) is cloned separately (Figure 1B). By treating the class sizes separately, competition at the cloning steps (i.e. ligation, transformation, and growth), which would normally select for smaller species, is eliminated. The removal of that competition increases representation of the longest transcripts. Additionally, the cloning technique used to create these libraries is extremely efficient for directional cloning of full-length cDNAs without blunting and ligating linkers onto the ends of the cDNA molecules. As a result, the cloning of genomic DNA or chimeric cDNAs from different transcripts is extremely rare.

This template is the perfect source to quickly clone any human gene, and is ideal for obtaining full-length cDNAs to be used for downstream expression analysis and functional studies (Figure 2).

TABLE 1**Messenger RNA sources represented in the MegaMan Human Transcriptome Library**

Human Tissues (32)	Human Cancer Cell lines (34)
Bone Marrow	29SR (Lymphoblast, Leukemia)
Brain, portion:	786-O (Renal, Adenocarcinoma)
Amygdala	A549 (Lung, Carcinoma)
Caudate Nucleus	ACHN (Renal, Adenocarcinoma)
Cerebellum	BT-549 (Mammary Gland, Carcinoma)
Corpus Callosum	CCF-STTG1 (Brain, Astrocytoma)
Hippocampus	CCRF-CEM (T Lymphoblast, Leukemia)
Insula	COLO 205 (Colon, Adenocarcinoma)
Medulla Oblongata	D341 Med (Brain, Medulloblastoma)
Nucleus Accumbens	DBTRG-05MG (Brain, Glioblastoma)
Paracentral Gyrus	DLD-1 (Colon, Adenocarcinoma)
Pons	DMS 114 (Lung, Carcinoma)
Postcentral Gyrus	DU 145 (Prostate, Adenocarcinoma)
Putamen	HCN-1A (Brain, Cortical neuron)
Subthalamic Nucleus	HCT-15 (Colon, Adenocarcinoma)
Thalamus	HCT-116 (Colon, Carcinoma)
Brain, whole	HL-60 (Promyeloblast, Leukemia)
Heart	Hs 578T (Mammary Gland, Carcinoma)
Kidney	K-562 (Bone Marrow, Leukemia)
Liver	M059K (Brain, Glioblastoma)
Lung	MCF7 (Mammary Gland, Adenocarcinoma)
Lymph Node	MDA-MB-231 (Mammary Gland, Adenocarcinoma)
Placenta	MDA-MB-435S (Mammary Gland, Carcinoma)
Prostate	MDA-MB-468 (Mammary Gland, Adenocarcinoma)
Skeletal Muscle	MOLT-4 (T Lymphoblast, Leukemia)
Small Intestine	NCI-H460 (Lung, Carcinoma)
Spinal Cord	OVCAR-3 (Ovarian, Adenocarcinoma)
Spleen	PC-3 (Prostate, Adenocarcinoma)
Stomach	PFSK-1 (Brain, Neuroectodermal tumor)
Testis	RPMI 7951 (Skin, Melanoma)
Thyroid	RPMI 8226 (B Lymphocyte, Leukemia)
Trachea	SHP-77 (Lung, Carcinoma)
Uterus	SW620 (Colon, Adenocarcinoma)
	T-47D (Mammary Gland, Carcinoma)

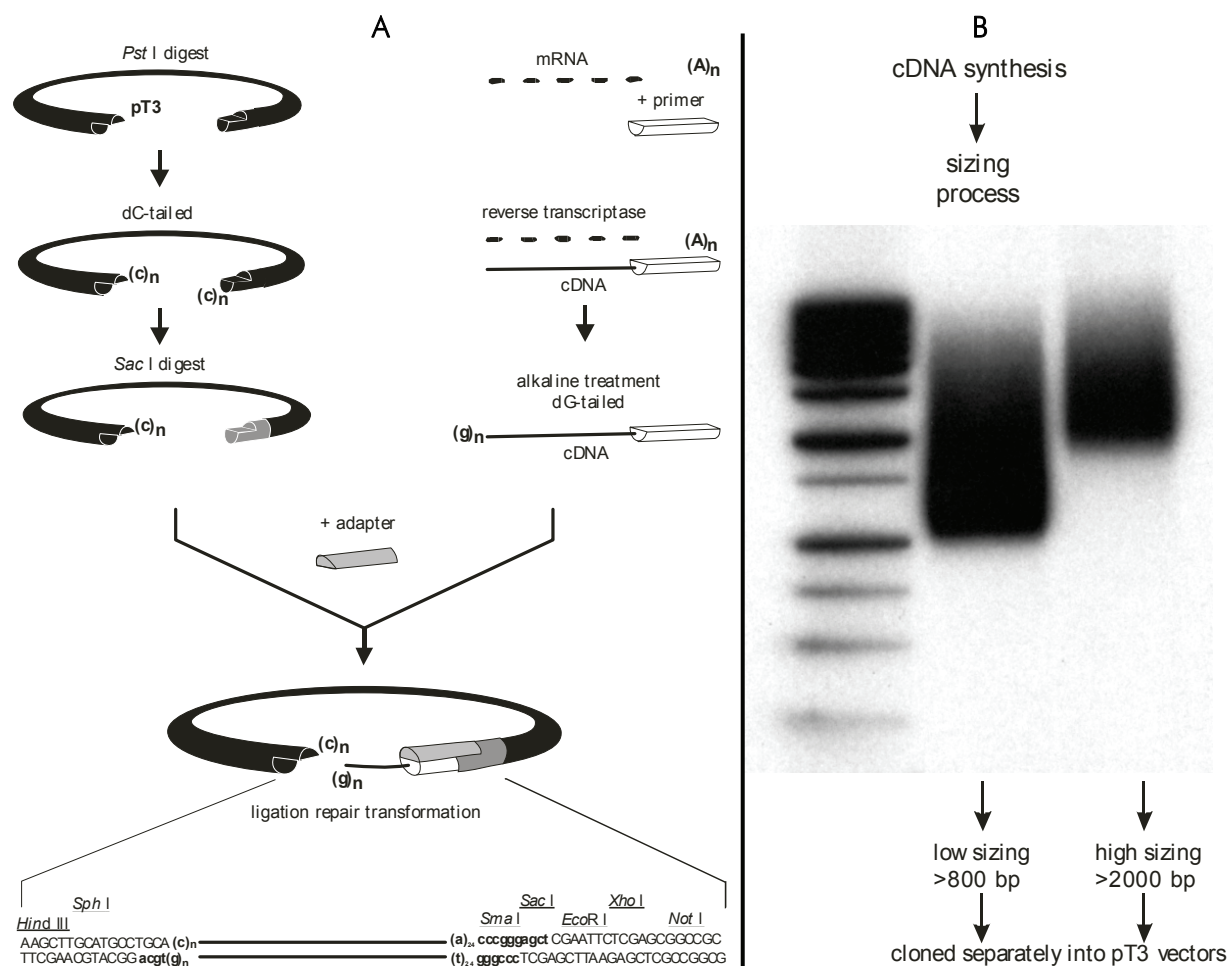


FIGURE 1 Cloning technique used to make the MegaMan Human Transcriptome Library (A) and size fractionation of cDNA used for library construction (B).

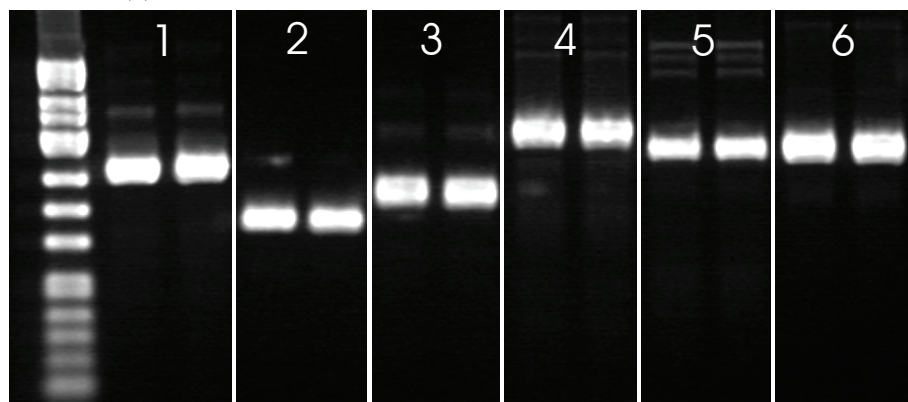


FIGURE 2 Successful amplification in duplicate of gene fragments using *PfuUltra* DNA Polymerase. Gene fragments from low (30-60 copies per cell), medium (300-600 copies per cell) and high abundance genes (>1500 copies per cell) were used to test representation in the MegaMan library. Medium abundance genes human ADP ribosylation factor 1 and human ADP ribosylation factor 3 are represented in lanes 1 and 2, respectively; high abundance genes human cytoskeletal β actin and human cytoskeletal γ actin in lanes 3 and 4, respectively; low abundance genes human protein phosphatase 1 and human ornithine decarboxylase in lanes 5 and 6, respectively. These data show that even the rarest mRNA transcripts, such as the ornithine decarboxylase gene transcript, have been preserved.

PROTOCOL

PCR Protocol

1. Prepare the amplification reaction by adding the following components to a PCR tube:

Notes *Acetamide and DMSO are optional components that can be added separately to reactions if less-than-satisfactory results are obtained using the standard protocol. Acetamide should be added to a final concentration of 5% (w/v) and DMSO should be added to a final concentration of 3% (v/v).*

We offer Stratagene PfuUltra HF DNA Polymerase for the highest fidelity amplification [Stratagene Catalog #600380, 600382, 600384].

Control Reaction

- 1.0 µl MegaMan human transcriptome library
- 0.4 µl dNTP mix (25 mM of each dNTP)
- 2.0 µl Control Primer Set
- X µl DNA polymerase
- 5.0 µl PCR reaction buffer (10×)
- X µl nuclease-free dH₂O
- 50 µl Total reaction volume

Experimental Reaction

- 1.0 µl MegaMan human transcriptome library
- 0.4 µl dNTP mix (25 mM of each dNTP)
- 2.0 µl gene-specific primers (10 µM of each primer)
- X µl DNA polymerase
- 5.0 µl PCR reaction buffer (10×)
- X µl nuclease-free dH₂O
- 50 µl Total reaction volume

Reaction Amplification

1. Amplify the DNA using the following PCR cycling conditions:

Number of Cycles	Temperature	Duration
1 cycle	94°C	3 minutes
35 cycles	94°C	45 seconds
	58°C	30 seconds
	72°C	1 minute/kb ^a
1 cycle	72°C	7 minutes

^a A one minute minimum is required for the 72°C elongation step.

Expected Control Amplification Results

Amplification of the control primer amplicon should result in a PCR product of 753 bp in length.

TROUBLESHOOTING

Observations	Suggestions
Poor amplification of low-abundance mRNAs	Increase the amount of MegaMan human transcriptome library to 2 µl per amplification reaction.
Low or no yield	Increase the number of PCR amplification cycles to 40.
	Amplify using either acetamide (5% final concentration) or DMSO (3% final concentration).
	Amplify using 2.5 U/reaction of <i>PfuUltra</i> HF DNA Polymerase
	Increase the amount of DNA Polymerase used per reaction (e.g. increase <i>PfuUltra</i> HF DNA Polymerase to 5.0 U/reaction).

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at <http://www.stratagene.com/MSDS/>. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

An Agilent Technologies Division

MegaMan Human Transcriptome Library

Catalog #790000 and 790001

QUICK REFERENCE PROTOCOL

PCR Protocol

- ♦ Prepare the amplification reactions by adding the following components to a PCR tube:

Notes *Acetamide and DMSO are optional components that can be added to reactions separately if less-than-satisfactory results are obtained using the standard protocol. Acetamide should be added to a final concentration of 5% (w/v) and DMSO should be added to a final concentration of 3% (v/v).*

Agilent offers Stratagene PfuUltra HF DNA Polymerase for the highest fidelity amplification [Stratagene Catalog #600380, 600382, 600384].

Control Reaction

1.0 µl MegaMan human transcriptome library
0.4 µl dNTP mix (25 mM of each dNTP)
2.0 µl Control Primer Set
X µl DNA polymerase
5.0 µl PCR reaction buffer (10×)
X µl nuclease-free dH₂O
50 µl Total reaction volume

Experimental Reaction

1.0 µl MegaMan human transcriptome library
0.4 µl dNTP mix (25 mM of each dNTP)
2.0 µl gene-specific primers (10 µM of each)
X µl DNA polymerase
5.0 µl PCR reaction buffer (10×)
X µl nuclease-free dH₂O
50 µl Total reaction volume

Reaction Amplification

- ♦ Amplify the DNA from the PCR protocol using the following cycling conditions:

Number of Cycles	Temperature	Length of Time
1 cycle	94°C	3 minutes
35 cycles	94°C	45 seconds
	58°C	30 seconds
	72°C	1 minute/kb ^a
1 cycle	72°C	7 minutes

^a A one minute minimum is required for the 72°C elongation step.

Expected Control Amplification Results

- ♦ Amplification of the control primer amplicon should result in a PCR product 753 bp in length.